

LISTING OF CLAIMS

1. (Currently amended) A method of isolating and identifying a microbial species from a source environment, comprising:

gathering from the source environment a sample suspected of containing ~~at least one~~ a microorganism that has not been cultured using standard culturing techniques;

providing a volume of culture medium to the ~~microorganism sample~~ in at least one microtiter plate compartment ~~in order to dilute the sample to a culture density of 1 to 10 cells per milliliter, wherein the culture medium comprises substrate concentrations that are similar to those of the source environment;~~

incubating the microorganism in the ~~culture~~ medium for a period of time and in an environment sufficient to result in growth of the microorganism if the ~~culture~~ medium and environment are capable of supporting such growth to produce a culture sample, ~~wherein growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about 5×10^4 cells/milliliter;~~

detecting growth of the microorganism using an automated detection method that comprises removing a portion of the culture sample and depositing the portion onto a surface, ~~wherein detecting growth of the microorganism consists of detecting an increase in a number of microorganisms in the compartment to no more than about 5×10^4 cells/milliliter, and wherein detecting growth of the microorganism indicates that the microbial species has been isolated from the source environment; and~~

identifying the microbial species, wherein identifying the microorganism ~~includes comprises~~ hybridization of a probe to a nucleic acid molecule of the microorganism; amplification of a nucleic acid molecule of the microorganism; immunodetection of a molecule of the microorganism; sequencing of a nucleic acid molecule of the microorganism; or a combination of two or more thereof.

2. (Original) The method of claim 1, wherein a plurality of individual microorganisms are separately incubated in microtiter plate compartments.

3. (Original) The method of claim 2, wherein the plurality is at least 20.

4. (Original) The method of claim 2, wherein the plurality is at least 50.

5. (Original) The method of claim 2, wherein the plurality is at least 100.

6. (Original) The method of claim 2, wherein the plurality is at least 400.

7. (Original) The method of claim 2, wherein the plurality is at least 1000.

8. (Original) The method of claim 2, wherein the plurality is at least 1500.

9-10. (Canceled)

11. (Original) The method of claim 1, wherein the source environment is a non-laboratory environment.

12. (Original) The method of claim 1, wherein the source environment is a natural environment.

13. (Original) The method of claim 1, wherein more than one microorganism is gathered from the source environment.

14. (Original) The method of claim 13, wherein each organism is provided a volume of medium in a separate compartment.

15. (Previously presented) The method of claim 14, wherein the volume of medium is no greater than about 1 ml.

16. (Original) The method of claim 14, wherein the organisms are placed in the separate compartments using flow cytometry, cell sorting, or dilution.

17. (Previously presented) The method of claim 1, further comprising counting at least one microorganism that grew.

18. (Previously presented) The method of claim 1, wherein identifying the microorganism includes hybridization of a probe to a nucleic acid molecule of the microorganism.

19. (Previously presented) The method of claim 1, wherein identifying the microorganism includes amplification of a nucleic acid molecule of the microorganism.

20. (Previously presented) The method of claim 1, wherein identifying the microorganism includes immunodetection of a molecule of the microorganism.

21. (Previously presented) The method of claim 1, wherein identifying the microorganism includes sequencing of a nucleic acid molecule of the microorganism.

22. (Canceled)

23. (Previously presented) The method of claim 1, wherein identification of the microorganism is automated.

24. (Original) The method of claim 17, wherein identifying or counting a microorganism comprises depositing cells in a two-dimensional array, such that different cultures arising from different cells each occupy a unique position in the array.

25. (Original) The method of claim 17, wherein identifying or counting a microorganism comprises use of a technique that reveals a genetic or enzymatic property of the microorganisms.

26. (Original) The method of claim 17, wherein a cultured strain of bacteria, called a reporter strain, is added to the medium with an unknown cell from nature, such that production

of at least one compound by the unknown cell is revealed by a growth or genetic responses of the reporter strain.

27. (Previously presented) The method of claim 1, wherein the detection method comprises removal of substantially all of the medium from the cultured sample.

28. (Previously presented) The method of claim 1, wherein identifying the at least one microbial species comprises sequencing a target nucleic acid sequence of the microbial species, and comparing the sequence of the target nucleic acid to at least one known sequence of the target nucleic acid from at least one known organism.

29. (Previously presented) The method of claim 28, wherein the target nucleic acid sequence is a ribosomal RNA sequence.

30. (Currently amended) A method of isolating and identifying a microbial species from a marine source environment, comprising:

gathering from the source environment a sample suspected of containing a plurality of microorganisms ~~that have not been cultured using standard culturing techniques~~;

providing a volume of culture medium based on sea water to the plurality of microorganisms in a plurality of microtiter plate compartments, in order to dilute the sample such that each compartment receives no more than about three microorganisms;

incubating the plurality of microorganisms in the medium for a period of time and in an environment sufficient to result in growth of the microorganism if the medium and environment are capable of supporting such growth to produce a plurality of culture samples;

detecting growth of at least one of the plurality of microorganisms using a detect method that comprises depositing a portion of the culture sample onto a surface using a filtration manifold, ~~wherein detecting~~ growth of the microorganism comprises an increase in the number of microorganisms in the compartment to no more than about 5×10^4 cells/milliliter, and wherein detecting growth of the at least one microorganism indicates that the microbial species has been isolated from the source environment; and

identifying the microbial species, using a method that comprises:

sequencing a ribosomal RNA sequence of the microbial species;
comparing the sequence of the ribosomal RNA to at least one known
ribosomal RNA sequence from at least one known organism; and
assigning an identity to the microbial species based on sequence similarity
to the ribosomal RNA of the known organism.

31. (New) The method of claim 1, wherein the sample gathered from the source
environment contains at least about 5 microorganisms.

32. (New) The method of claim 1, wherein the sample gathered from the source
environment contains at least about 10 microorganisms.

33. (New) The method of claim 1, wherein the sample gathered from the source
environment contains at least about 20 microorganisms.

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